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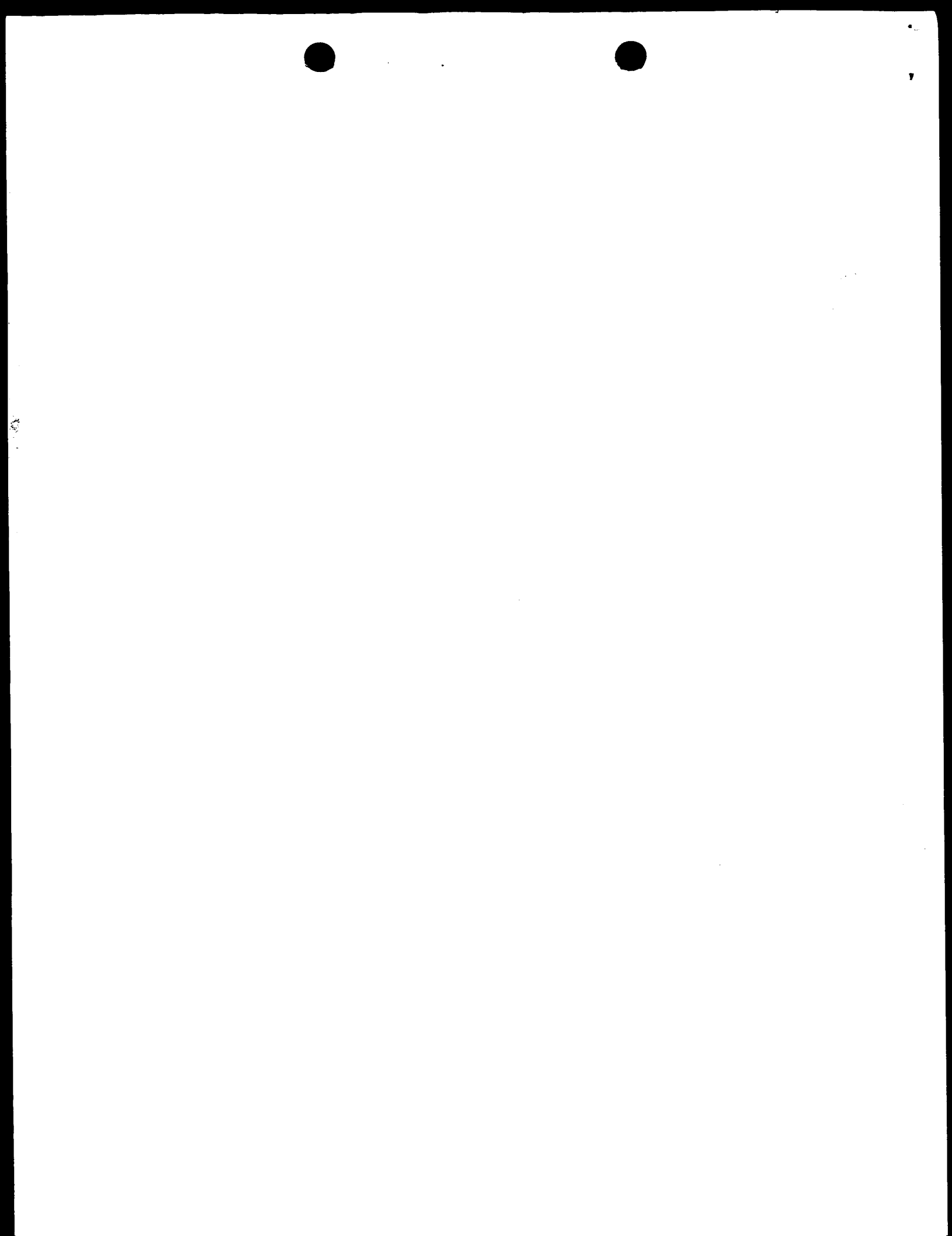
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## Patents Form 1/77

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## 2. Patent application number

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9703670.1

21 FEB 1997

## 3. Full name, address and postcode of the or of each applicant (underline all surnames)

The University of Sheffield  
Western Bank  
**SHEFFIELD**  
S10 2TN

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

511242052

## 4. Title of the invention

MONONUCLEAR PHAGOCYTES IN THERAPEUTIC  
DRUG DELIVERY

## 5. Name of your agent (if you have one)

"Address for service" in the United Kingdom  
to which all correspondence should be sent  
(including the postcode)

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Patents ADP number (if you know it)

2253003

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Country

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Number of earlier application

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- a) any applicant named in part 3 is not an inventor, or  
b) there is an inventor who is not named as an applicant, or

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Claim(s)

Abstract

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Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)Request for preliminary examination and search (*Patents Form 9/77*)Request for substantive examination (*Patents Form 10/77*)Any other documents  
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William Jones <sup>dep</sup>

Date

WILLIAM JONES (YORK)

21.02.1997

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ROBERT C DOCHERTY (Dr)

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**MONONUCLEAR PHAGOCYTES IN**  
**THERAPEUTIC DRUG DELIVERY**

- 5     The invention relates to a method of drug delivery; means therefor including components thereof which have particular, but not exclusive, application in cancer therapy development.

10     Macrophages often comprise 20-60% of the tumour cell mass in breast carcinomas and form intimate contacts with malignant cells. This has long been thought to represent part of the host's defence mechanisms against the tumour; however, their function at such sites in the body remains an enigma at present as macrophages isolated from human or murine tumours exhibit reduced tumouricidal, phagocytic and antigen-presenting activities compared to those from normal tissues (1).

15     Monocytes are produced in the bloodstream and extravasate (i.e. exit) into surrounding tissues including such diseased tissues as malignant tumours and atherosclerotic plaques, where they differentiate into macrophages and perform immune, secretory, phagocytic and other functions. Monocytes and macrophages are collectively termed mononuclear phagocytes. As tissue  
20     macrophages have a lifespan of 60 to 90 days and the number of macrophages in tumours remains constant, it is believed that there is a constant attachment of monocytes to the tumour endothelium and influx of monocytes into the tumour cell mass.

25     diseased tissue (e.g. malignant tumours, ischaemic heart tissue etc). Hypoxia

and/or hypoglycaemia is thought to occur in growing tumours when the increasing metabolic demands of the rapidly expanding tumour cell population outstrip the supply of oxygen/glucose etc., made available to them by simple diffusion across the tumour mass from vessels in surrounding normal tissues.

Our recent and surprising data indicate that once monocytes enter a tumour from the bloodstream, they rapidly differentiate into macrophages and preferentially congregate in hypoxic (i.e. poorly vascularised and necrotic) sites deep within a tumour mass remote from blood vessels. Refer to Figure 1, which represents a bar chart of the Distribution of Macrophages in Relation to Blood Vessels. Moreover, breast tumours, with more hypoxic/necrotic areas, are more heavily infiltrated with macrophages, which preferentially locate to, or around, the necrotic sites (refer to Figure 2, which represents a bar chart of the Association of Macrophage Index with Necrosis in Breast Carcinomas). Experimental hypoxia has been shown to induce the production of angiogenic factors by macrophages *in vitro* (2). Taken together these data could underpin our recent finding, that increased numbers of macrophages in breast tumours equate with higher levels of angiogenesis and increased fatalities in breast cancer (3).

We examined tumour biopsies from 100 breast cancer patients, and found that macrophage infiltration was strongly associated with reduced relapse-free interval and overall survival - even in lymph node negative patients alone (i.e. the better prognosis group). Indeed macrophage infiltration proved to be almost as important in predicting outcome as lymph node status in breast cancer. We went on to see if this was due to an effect of macrophages on such important parameters of tumour aggressiveness as the mitotic index,

receptor status, degree of angiogenesis, etc. It was at this point that a highly significant and unexpected correlation between the degree of macrophage infiltration within a tumour mass and angiogenesis emerged, suggesting a role for this cell type in some or all of the steps of tumour angiogenesis (3).

- 5 Our unique observations suggest that, since the entry or presence of macrophages into or in such diseased tissues appears to be deleterious to the patient, therapies specifically focused on either blocking their entry, their destruction and/or their exploitation to carry therapeutic agents into such diseased tissues could prove to have therapeutic benefits.
- 10 Monoclonal antibodies have been considered for many years to be the best way of delivering cytotoxic agents to tumours, but this approach has so far been disappointing in clinical trials (4, 5). The main reason for the lack of therapeutic efficacy of antibody conjugates in solid tumours is the resistance of the tumour to penetration by macromolecules. In studies with
- 15 radiolabelled antibodies, typically only 0.001-0.01% of the injected dose localises to each gram of solid tumours in humans (6, 7). The poor penetration of antibodies is thought to be due to a number of factors. Firstly, the antibodies must cross the physical barrier of the endothelial cell layer in tumour blood vessels and the, often dense, fibrous stroma packed
- 20 between tumour cell areas. Secondly, the dense packing of tumour cells and tight junctions between epithelial tumour cells hinder the transport of the antibody within the tumour mass. Thirdly, the absence of lymphatics within the tumour contributes to the build up of a high interstitial pressure which
- 25 One solution to the poor penetration of antibody conjugates into solid

tumours would be to attack the endothelial cells in the tumour, instead of the tumour cell themselves, which are readily accessible to intravenously injected antibody. This then leads to destruction of the tumour blood vessels and the death of neighbouring tumour cells which rely on the blood supply for oxygen and nutrients. Early studies using murine models have met with some success in this area (8), but there remains the problem in humans of how to target tumour endothelial cells and not those in normal tissues. Furthermore, as mentioned earlier, our work strongly suggests the tumour necrosis resulting from this approach will trigger compensatory angiogenic activities in tumour-infiltrating macrophages in the vicinity. This would oppose the effects of the therapeutic agent.

Our inventive solution is to attach the agent (e.g. tumour or endothelial cell cytotoxin) to monocytes in the peripheral blood, that is the cells which gain entry to the tumour in large numbers to form tumour-infiltrating macrophages. Since monocytes swarm to the tumour site in large numbers as an early and ongoing event in tumour development, they can be used to carry therapeutic agents (e.g. cytotoxic drugs or toxins) into the centre of solid tumours. In support of this suggestion is the fact that many studies have shown that malignant tumours actively recruit this cell type and that monocytes then follow a chemotactic trail produced by distressed (i.e. hypoxic/necrotic) tumour cells. Further, we have uniquely shown that mononuclear phagocytes congregate in tumour areas where they are most needed, i.e. hypoxic areas, possibly to help initiate angiogenesis.

Although we have described the invention with particular reference to tumour cells, it can be used in any instance where mononuclear phagocytes infiltrate or are attracted to hypoxic tissue or conditions. Thus, the invention can be



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used during development to control the vascularisation of developing tissue, typically, but not exclusively, with a view to targeting a hypoxia regulatable agent so as to promote or enhance vascularisation. Alternatively the invention can be used to target hypoxia regulatable agents to damaged tissue, for example to tissue where de-vascularisation has occurred following damage to the vascular system via an amputation, stroke, cardiac arrest, extreme hypertension, ischaemia, burns etc.

It follows from the information provided herein that the invention may be used to prevent or reduce tissue vascularisation, or to promote or enhance vascularisation, or to simply deliver selected drugs to hypoxic sites where mononuclear phagocytes are typically present.

It is therefore an object of the invention to provide a novel drug delivery system which exploits the fact that mononuclear phagocytes collect or are attracted to hypoxic sites.

It is yet a further object of the invention to provide a regulatable drug delivery system which enables the activation of said drug to be controlled, and ideally but not exclusively, to be controlled so as to only be active under hypoxic conditions.

It is yet a further object of the invention to provide a novel drug for use in the drug delivery system of the invention.

ADVOCATES OF THE INVENTION ARE PARTICULARLY INTERESTED IN THE USE OF THE INVENTION IN THE TREATMENT OF HYPOTENSIVE STATES.

According to a first aspect of the invention there is therefore provided a therapeutic composition comprising a regulatable agent and/or an agent that binds to a cell surface element of a mononuclear phagocyte.

In a preferred embodiment said agent is a hypoxia regulatable agent.

- 5 It will therefore be apparent that the hypoxia regulatable agent will be affected by hypoxic conditions and typically affected so as to only be active in such conditions. Moreover, said binding agent, which is typically coupled to said regulatable agent, attaches the composition to mononuclear phagocytes and so targets the regulatable agent, to sites typically infiltrated by  
10 mononuclear phagocytes. Thus in the instance where said mononuclear phagocytes penetrate hypoxic sites said composition is suitably delivered to such sites and the regulatable agent becomes active.

- The invention is elegant in so far as the body's own mechanisms are exploited for the specific delivery of drugs but the invention is safe in so far as the  
15 drugs remain inactive until exposed to hypoxic conditions.

Given the above nature of the invention agents suitable for use in manufacturing the said composition will be known to those skilled in the art and therefore the following preferred embodiments are not intended to be exhaustive but rather illustrative.

- 20 For example, in one embodiment of the invention said hypoxia regulatable agent may comprise a therapeutic gene, that is to say a gene encoding a therapeutic agent which is under the control of a hypoxia sensitive agent such as a hypoxia regulated expression element i.e. a promoter or enhancer which

is sensitive to hypoxia. Thus, under conditions of hypoxia said element will be activated so as to enable the gene encoding for the therapeutic agent to be expressed. In this embodiment of the invention the binding agent is optional, and indeed, may be substituted for an agent that ensures internalisation of said therapeutic gene with a view to incorporating same into the mononuclear phagocyte genome.

In addition, or alternatively, the hypoxia regulatable therapeutic gene may encode a prodrug activation enzyme, that is to say an enzyme which converts a relatively inactive drug into a more active one. An example of this kind of enzyme is thymidine phosphorylase which activates the 5-FU prodrugs capcetabine and furtulon. An other example of a pro-drug activation enzyme is the herpes simplex thymidine kinase or cytosine deaminase which, once internalised into the mononuclear phagocytes would act as a reservoir for activation of the prodrugs ganciclovir and 5-fluorocytosine.

Other examples of hypoxia regulatable therapeutic genes are to be found in PCT/GB95/00322.

In yet an alternative embodiment of the invention said agent may be regulated by means other than hypoxia.

Indeed, the use of hypoxia to regulate the expression/activity of a therapeutic gene may not be an exclusive feature of the utilisation of mononuclear phagocytes as a means of targeting therapeutic genes to tumours or regions

In a further embodiment it may be preferable to place the therapeutic gene

under the control of an inducible or repressible promoter element, transcription from which can be modulated to regulate the effective dose of the therapeutic gene. This may result in more accurate control of expression of the therapeutic gene and thereby reduce undesirable side effects as a consequence of the gene therapy.

By way of example, a constitutively active promoter used to express a therapeutic gene/agent could be placed under the regulation of the antibiotic tetracycline by inclusion in the promoter of the tetracycline repressor DNA sequence. The vector DNA incorporating the therapeutic promoter cassette could include the DNA sequence of the tetracycline repressor protein under the control of a suitable promoter element. Transfection of said DNA construct into the mononuclear phagocytic genome would result in expression of tetracycline repressor protein and a repression in the transcription of the therapeutic gene. Transfected mononuclear phagocytes could then be re-introduced into the patient to allow said phagocytes to infiltrate, for example, a tumour. Tetracycline could then be administered either orally, via an intravenous route or injected directly into the tumour tissue. The administration of tetracycline to the patient would result in inactivation of the binding between the tetracycline repressor protein and the operator DNA sequence in the promoter of the therapeutic gene thus enabling its expression. The effective dose of tetracycline can be varied to regulate the expression of the therapeutic gene/agent and thereby optimise the effect of the therapeutic gene/agent.

The system may also be exploited to enable the regulated expression of a "suicide" gene, the expression of which in a mononuclear phagocyte would result in the death of the phagocyte carrying the gene. Selective removal of

mononuclear phagocytes may then inhibit revascularisation of tumour tissue thereby restricting tumour development.

5 This example of regulated expression has been shown to occur in both prokaryotic (WO 9532295-A) and eukaryotic cells (9, 10) to successfully regulate the expression of both homologous and heterologous DNA sequences. This description is merely to serve as an example of how a prokaryotic sequence can be used to regulate the expression of a eukaryotic gene and is not meant to be exclusively limited to the tetracycline repressor operon.

10 In a yet further alternative embodiment said regulatable agent may comprise a bio-reductase prodrug such as RSU 1069 which is activated at very low levels of oxygen as well as with contact with enzymes such as reductases. Thus, where not only hypoxia, but protein-protein interaction, typically enzymic, is required for activation of said regulatable agent, said therapeutic  
15 composition of the invention may further comprise an agent that activates said regulatable agent, such as a reductase.

Alternatively, or in addition, especially where internalisation of said therapeutic composition is required said therapeutic composition may further comprise an internalisation agent so as to ensure that the therapeutic  
20 composition is internalised by the mononuclear phagocytes. Agents which are suitable for ensuring internalisation of the therapeutic composition include, but are not limited to, plasminogen activation inhibitors (PAI-1 or

or yet a further preferred embodiment of the invention said binding agent is

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adapted to bind to any one or more cell surface mononuclear phagocyte molecules such as antigens or receptors. Further, said binding agent may comprise an antibody to any one or more of said molecules such as antigens or receptors, or an effective fragment of said antibody. Alternatively still said  
5 binding agent may comprise a suitable ligand either synthetically manufactured or naturally occurring.

A brief list of those cell surface molecules that may be targeted by said binding agent is as follows; the receptor for human Urokinase Plasminogen Activator (uPAR; CD87); the receptor for human Colony Stimulating Factor  
10 (CSF-1); CD63; CD64; CD11b; CR3; the scavenger receptor; all or part of the receptor for the various forms of human monocyte chemoattractant protein (MCP-1, 2, etc); CD14; mannose or mannose-6-phosphate surface receptors; CD16; or HLA-DR.

According to a yet further aspect of the invention there is provided a delivery  
15 system for targeting therapeutic compositions to hypoxic sites comprising a regulatable agent and an agent for controlling the functional effectiveness thereof, and coupled thereto, a binding agent for a cell surface molecule of a mononuclear phagocyte.

According to a yet further aspect of the invention there is provided a method  
20 for targeting desired agents to hypoxic sites comprising;

- (i) coupling at least one of said agents to a binding agent for a cell surface molecule expressed by a mononuclear phagocyte;
- (ii) exposing said coupled agents to mononuclear phagocytes; and

(iii) allowing said mononuclear phagocytes to migrate, under conditions that support migration, either *in vitro*, *in vivo* or *ex vivo*.

According to a yet further aspect of the invention there is provided a method for treating conditions associated with hypoxic states comprising  
5 administering to an individual to be treated the therapeutic composition of the invention.

According to a yet further aspect of the invention there is provided a method for treating conditions associated with hypoxic states comprising withdrawing blood and/or serum from an individual to be treated and treating said blood  
10 and/or serum *in vitro* with an inducible therapeutic gene or fragment or part thereof under conditions that enable incorporation of said gene into the genome of mononuclear phagocytes and re-injecting said treated blood and/or serum into the individual either systemically or directly into a hypoxic area.

According to a yet further aspect of the invention there is provided  
15 mononuclear phagocytes which have coupled thereto, or internalised therein, a regulatable agent and an agent that is adapted to bind to a mononuclear phagocyte ligand which is typically found on the cell surface of said mononuclear phagocyte.

According to a yet further aspect of the invention there is provided a method  
20 for selectively destroying a mononuclear phagocyte comprising attaching thereto or internalizing therein a cytotoxic, hypoxically activated agent and exposing said mononuclear phagocyte to said agent.

In the instance where hypoxia occurs/exists naturally said mononuclear phagocyte migrates in a normal manner to said hypoxic area so that the said agent is only activated at a target area. In this way the potentially deleterious effects of mononuclear phagocytes in tumours is obviated. Moreover, having  
5 regard to the nature of said agent a bystander effect may be achieved, for example where said cytotoxic agent is released on death of said mononuclear phagocyte it may have a further deleterious effect on the hypoxic tissue, such as, but not limited to, tumour tissue.

Many of the preferred embodiments hereinbefore described represent  
10 appropriate modifications of any one or more of the above referred to further aspects of the invention.

An embodiment of the invention will now be described by way of example only with reference to the following Table and figures wherein:

Table 1 represents specific examples of drug conjugates;

15 **Materials and Method**

*Drug Delivery*

The drug conjugate of choice can be infused (repeatedly or as a single injection) into the general circulation so as to bind *in vivo* to the surface of systemic mononuclear phagocytes and/or macrophages already resident in  
20 diseased tissues (e.g. malignant tumours). In support of the aforementioned mode of drug delivery is the finding that when a monoclonal antibody, specific for the Mac-1 antigen on macrophages, is conjugated to indium (a



radioactive element) and injected intravenously into mice bearing a solid tumour, it was seen on scintographs to accumulate predominantly in the cancer lesion (11). Alternatively the drug conjugate can be exposed to monocytes *ex vivo*, following their purification from the blood of patients using such standard methods as Ficoll- Hypaque gradients and elutriation as described previously in (12).

Homing of blood monocytes loaded up with drug conjugates into malignant tumours can be augmented by prior treatment with conventional systemic therapies which induce local inflammation/necrosis in the diseased tissue (e.g. radiotherapy or chemotherapy in the case of cancer patients). This stimulates the release of chemoattractant factors for monocytes/macrophages such as MCP-1 (13, 14) and would thus enhance the delivery and hence the therapeutic efficacy of the drug conjugate at the diseased site.

*Mode of production of selected drug conjugates*

Drug 1. (with reference to Table 1) *RSU1069 - F(ab)<sub>2</sub> of a monoclonal antibody to CD87 (uPAR)*

This conjugate uses a highly specific F(ab)<sub>2</sub> fragment a monoclonal antibody to CD87 (urokinase plasminogen activator receptor; uPAR) to target naturally occurring uPAR on the surface of monocytes and macrophages.

A monoclonal antibody to CD87 is made as described in (15) and then raise the antibody (i.e. as the antigen), the epitope for the antibody generated

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may either be in the (i) ligand (i.e. uPA) -binding portion of the uPAR (in which case the drug conjugate will only bind to unoccupied uPAR on monocytes/macrophages), or (ii) the non ligand (i.e. uPA) -binding portion of the uPAR (in which case the drug conjugate will bind to both unoccupied and unoccupied uPAR on monocytes/macrophages). The most effective drug uptake is likely to be achieved using the latter form of conjugate.

The fragment of the CD87 monoclonal antibody is conjugated to the bioreductive prodrug, RSU1069, by the latter being reacted with an agent such as arylazide to add a N-hydroxysuccinimide group. This is then cross-linked at neutral pH to the CD87 antibody fragment via amine groups to form a conjugate. This method is well established for conjugating drugs to proteins and is described fully in (16).

Drug 2. (with reference to Table 1) i.e. *RSU1069 - PAI-2*

This conjugate uses the affinity of plasminogen activator inhibitor 2 (PAI-2) for urokinase plasminogen activator receptor (uPAR) - urokinase plasminogen activator complexes to target the bioreductive prodrug to the surface of monocytes and macrophages. PAI-2 triggers the internalization of uPAR-uPA complexes, so the internalization by these cells of the bioreductive prodrug attached to PAI-2 is assured.

Naturally occurring PAI-2 is obtained from the culture supernatant of human blood monocytes stimulated maximally with interleukin 1 or 2 as described in (17). This is then purified to homogeneity in the usual manner by elution from an anti-PAI-2 immunoaffinity column. Alternatively, PAI-2 can be produced in a recombinant expression system and purified according to the

15

method of (18). The PAI-2 preparation is then conjugated to RSU1069 using the method outlined as hereinbefore described for drug 1(16).

Drug 6. (with reference to Table 1) i.e. Interleukin-2 (IL-2) gene linked to hypoxia responsive promoter.

- 5 This gene is transferred to monocytes/macrophages using a replication-defective adenoviral vector. Efficient transfer of genes into human macrophages has been achieved with this method with expression of the gene in 40-80% of the cells exposed to the vector and lasting up to 3 weeks after gene transfer (12).
- 10 Defective retroviral vectors, direct DNA internalization or such non-gene viral gene transfer systems can also be used such as cationic lipids, liposomes, lectins or polymers. Genes other than IL-2 which could be of therapeutic benefit include such other immunomodulators as  $\text{TNF}\alpha$ , or interferon gamma, prodrug activating enzymes, enzyme inhibitors, tumour antigens (to provoke
- 15 the hosts immune reactivity to the tumour) and anti-oncogenes (antibodies or antisense RNA). It will be understood by those skilled in the art that the DNA construct used may, embody a number of these genes rather than just one and is not intended to limit the scope of the application.

- 20 The hypoxically inducible expression control sequence (promoter) for the Epo or PGK genes (or multiple copies thereof) is/are coupled to one or more of the gene sequences of choice (e.g. IL-2 gene sequence) as described by one of us previously in (10).

Gene transfer

The method outlined in (12) involves incubating (under sterile conditions) freshly isolated blood monocytes or monocyte-derived macrophages (monocytes incubated overnight in teflon bags or on plastic cultured wells) in the presence of 100 plaque-forming units per cell of the purified replication-defective vector, Ad.RSV $\beta$ , harbouring the hypoxia-responsive promoter-IL-2 gene construct in RPMI incubation medium (1ml/10<sup>6</sup> cells). Gene transfer may be enhanced by simultaneous treatment of the cells with 100U/ml of human interferon gamma. Cells are then washed to remove free viral particles and interferon gamma and reincubated at 37°C in fresh RPMI medium in teflon bags. The same adenoviral vector but harbouring the E.coli  $\beta$ -galactosidase gene (Ad.RSV. $\beta$ gal) instead of the hypoxia-responsive promoter-IL-2 gene construct is used as a reporter gene (i.e. to check the efficiency of this gene transfer method to monocytes/macrophages *ex vivo*). The presence of the  $\beta$ -gal enzyme in cells after infection with Ad.RSV. $\beta$ gal is then assessed using histochemical methods as described in (19). The transfected cells (10<sup>8</sup> to 10<sup>9</sup> cells) are then injected sterile back into the bloodstream or directly into the appropriate diseased tissue (e.g. malignant tumour) of the donor as in (12).

*In vivo gene transfer:*

This is performed according to the *in vivo* method of adenoviral gene delivery described in (20). This involves injecting the vector (i.e. Ad.RSV $\beta$ ) bearing the hypoxia-responsive promoter-IL-2 gene construct into the bloodstream (primarily to label monocytes) and/or into the diseased tissue (at 10<sup>9</sup> to 10<sup>7</sup> plaque-forming units) to label tissue macrophages.

### Results

The results are represented as exemplary drug conjugate candidates in Table 1. It will be understood by those skilled in the art that such conjugates represent selected examples and are not intended to limit the scope of the invention, furthermore it will be understood that indeed any one example of a candidate Part I drug conjugate may be used in conjunction with any one example of a Part II candidate and/or any example of a Part III candidate. Additionally it will be understood that any example of a Part III candidate may be used in conjunction with any example of a Part II and/or Part I candidate drug conjugate.

The invention hereinbefore described therefore represents a most elegant and effective means and method of delivering a therapeutic and/or cytotoxic agent to a hypoxic site by use of monocytes and/or macrophages and their natural ability to congregate at a hypoxic site.

TABLE 1 - SPECIFIC EXAMPLES OF DRUG CONJUGATES

	<u>Part I</u>	<u>Part II</u>	<u>Part III</u>
	<u>Drug 1</u>	RSU1069 F(ab)2 to CD87 (uPAR)	-
5	<u>Drug 2</u>	RSU1069 PAI-2 (binds to receptor bound urokinase plasminogen activator (uPAR) & ensures internalization of drug)	-
10	<u>Drug 3</u>	RSU1069 PAI-2 (binds to receptor bound uPAR & ensures internalization of drug)	Cyt. P450  (reductase for activation of RSU1069 under hypoxia)
15	<u>Drug 4</u>	RSU1069 F(ab)2 to CSF-1 receptor (binds to human CSF receptor on surface of monocyte/ macrophages)	Cyt. P450
20	<u>Drug 5</u>	Cytosine deaminase (prodrug activating enzyme)	F(ab)2 to CD63  -

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**Drug 6**

*Gene for Inter-  
leukin 2  
(immuno-  
stimulatory  
cytokine) in  
Ad.RSV*

*Hypoxia-resp.  
promoter  
sequence*

5

**Drug 7**

*DNA sequence  
for soluble  
domain of VEGF  
receptor (eg. flk-1)  
in Ad. RSV*

*Hypoxia-resp.  
promoter  
sequence*

10

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